United States
Environmental Protection
Agency

Solutions to Analytical Chemistry Problems with Clean Water Act Methods

March 2007

Appendices
A) Text of October 26, 1984 Preamble, pp. 7-11
B) Text of Preamble to March 12, 2007 Methods Update Rule (Note flexibility only applies to 40 CFR Part 136 methods)
C) Recommended Approved Modifications to EPA Method 625

31. The rule approves the use of newer versions of 19 methods published by AOAC-International. The new versions of these methods are published in Official Methods of Analysis of AOAC-International, 16th Edition, 1995.

32. The rule approves the replacement of the mercuric sulfate catalyst with copper sulfate in methods approved for the determination of total Kjeldahl nitrogen (TKN).

33. The rule approves the use of styrene divinyl benzene beads and stabilized formazin as alternatives to the presently approved formazin standard for determination of turbidity.

34. As described in the preamble to the April 2004 proposed rule (69 FR 18213), EPA is adopting a new §136.6 to introduce greater flexibility in the use of approved methods. The section describes the circumstances in which approved methods may be modified and the requirements that analysts must meet to use these modified methods in required measurements without prior EPA approval. The rule also includes language at §136.6 (c) to clarify that analysts need only meet method performance requirements for target analytes (those analytes being measured for NPDES reporting) when using multi-analyte methods for compliance monitoring purposes. The rule also includes the language at §136.6 (d) to allow explicitly the use of capillary (open tubular) GC columns with EPA Methods 601-613, 624, 625, and 1624B as alternatives to the packed GC columns specified in those methods, provided that analysts generate new retention time tables with capillary columns to be kept on file with other information for review by auditors.

35. The rule withdraws 109 methods contained in EPA's "Methods for the Chemical Analysis of Water and Wastes" for which approved alternatives published by voluntary consensus standards bodies (e.g., ASTM and Standard Methods) are available.

Note: This flexibility applies only to Part 136 methods.

10. Section 136.6 is added to Part 136 to read as follows: PR 175-180

§ 136.6 Method Modifications and Analytical Requirements.

(a) *Definitions of terms used in this Section.*

(1) Analyst means the person or laboratory using a test procedure (analytical method) in this Part.

(2) Chemistry of the Method means the reagents and reactions used in a test procedure that allow determination of the analyte(s) of interest in an environmental sample.

(3) Determinative Technique means the way in which an analyte is identified and quantified (e.g., colorimetry, mass spectrometry).

(4) Equivalent Performance means that the modified method produces results that meet the QC acceptance criteria of the approved method at this part.

(5) Method-defined Analyte means an analyte defined solely by the method used to determine the analyte. Such an analyte may be a physical parameter, a parameter that is not a specific chemical, or a parameter that may be comprised of a number of substances. Examples of such analytes include temperature, oil and grease, total suspended solids, total phenolics, turbidity, chemical oxygen demand, and biochemical oxygen demand.

are met. When changing from a packed column to a capillary column, retention times will change. Analysts are not required to meet retention time specified in the approved method when this change is made. Instead, analysts must generate new retention time tables with capillary columns to be kept on file along with other startup test and ongoing QC data, for review by auditors.

(2) *Increased sample volume in purge and trap methodology.* Use of increased sample volumes, up to a maximum of 25 mL, is allowed for an approved method, provided that the height of the water column in the purge vessel is at least 5 cm. The analyst should also use one or more surrogate analytes that are chemically similar to the analytes of interest in order to demonstrate that the increased sample volume does not adversely affect the analytical results.

Part 141 – National Primary Drinking Water Regulations

11. The authority citation for Part 141 continues to read as follows:

Authority: 42 U.S.C. 300f, 300g-1, 300g-2, 300g-3, 300g-4, 300g-5, 300g-6, 300j-4, 300j-9, and 300j-11.

12. Section 141.21 is amended by adding four sentences to the end of footnote 1 to the Table in paragraph (f)(3) to read as follows:

§141.21 Coliform sampling.

regulatory framework for the specific limits is being promulgated as final.

Table ID contains the 67 parameters included under the general "pesticides" parameter in the 1976 Guidelines. Although most pesticides are organic compounds, they have been listed separately in Table ID rather than with the other organic parameters in Table IC because of the wide association between this subset of organic compounds and their end use. Sixteen of the 67 parameters are priority pollutants. Three additional pesticides were identified as priority pollutants under the consent decree. Table ID therefore now identifies 70 specific pesticides, of which 19 are priority pollutants. Methods 608 and 625, which were proposed for the priority organic toxic pollutants, were revised to incorporate substantive comments. All other references in Table ID have been updated, but the updated references do not require any substantive changes from previously approved test procedures.

Table IE now includes the five radiological test procedures approved in the 1976 Guidelines. All references have been updated, and an EPA reference has been added. There are no substantive textual changes in these updated test procedures.

B. GC, HPLC and GC/MS Test Procedures

Analyses for organics depend upon a variety of chromatographic techniques. See subsection III–B above. EPA proposed and is approving two HPLC methods (605 and 610), 10 GC methods, and three GC/MS methods (613, 624, and 625). In addition, EPA has responded to critiques of Methods 624 and 625 by approving two GC/MS/isotope dilution variants (1624 and 1625). Each method is accompanied by a specific set of quality assurance (QA) procedures. The QA process relies on specific control limits calculated for each parameter for which the method can be used. The control limits indicate the outer range of precision and accuracy found in an extensive inter-laboratory study. The limits represent the minimum threshold of quality expected of competent laboratories: 95 percent confidence level per compound for the 600 series and the 99 percent confidence level across the set of compounds for the 1624 and 1625 methods. Most analyses should have far better precision and accuracy. The calculations of specific numerical control limits for the calibration and quality control sections of the GC, HPLC, and GC/MS test procedures is interim final. This means that they are legally effective, but that EPA will accept comments on their calculation. All other parts of these test procedures are finally approved for the analysis of the parameters which are indicated in Tables IC and ID.

Each method is approved for specific organic compounds. In general, GC Methods 601–603 and GC/MS Methods 624 and 1624 are approved for the analyses of the purgeable priority pollutants. GC Methods 604 and 606–612 and GC/MS Methods 625 and 1625 are approved for the analysis of the non-purgeable, volatile priority pollutants, including, for Method 625 only, the priority pesticide pollutants. Method 625 is also approved for screening samples for 2,3,7,8-TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), but only GC/MS Method 613 is approved for final qualitative confirmation or quantification of 2,3,7,8-TCDD in samples. HPLC Methods 605 and 610 are also approved for the analysis of the nonpurgeable volatiles (the benzidines and polynuclear aromatic hydrocarbons). Methods 1624 and 1625 are approved for use interchangeably with the other test procedures which are being approved for the analysis of the priority toxic organic pollutants. Their most significant difference from Methods 624 and 625 is the requirement that, where available, stable, isotopically-labeled analogs of the priority pollutants are to be used as method internal standards. Since Methods 624 and 625 do permit flexibility in the selection of internal calibration standards and surrogate standards, Methods 1624 and 1625 are, in essence, acceptable variants permitted by Methods 624 and 625. They improve on Methods 624 and 625 and are generally preferable. However, Methods 624 and 625 are also being approved because they are widely available, slightly less expensive, and they are of use when interference and recovery efficiency are not expected to be problems.

In general, both GC/MS and non-MS test procedures have been approved for each of the priority toxic pollutants. Most of the revisions of the proposed test procedures were made either for clarification or to give the analyst more flexibility to practice professional judgment. These procedures now contain a section on safety, cautioning analysts of the potential hazards associated with exposure to the chemical reagents required by the test procedures, or to the toxic chemicals being analyzed. Recommended and mandatory quality assurance practices are also given in each of the test procedures.

Methods 601–604, 606–609, 611–613, 624, 625, 1624, and 1625 include specifications for performing the tests. These specifications are based on a required primary GC column and specified detector. A primary HPLC column and specified detector are required for Methods 605 and 610 and specifications are provided. The primary column is also used to identify the pollutant. A secondary column and detector are also defined, but not required, for non-MS Methods 601–604 and 606–611. The secondary column and detector can be used for confirmation of priority pollutants identified by the primary column for unfamiliar (non-routine) samples (see sections 1.2 of the methods). The GC/MS test procedures are suggested as the confirmatory test for identifications made by Methods 605 and 612, and may also be used as the confirmatory test for identifications made by Methods 601–604 and 606–611. For example, an unfamiliar sample which would be likely to need confirmation would be a single sample taken for an NPDES application. See 40 CFR 122.21. In contrast, routine monitoring, such as that for discharge monitoring reports, would be less likely to require a secondary column for confirmation since the sample is more likely to be familiar to the analyst.

Methods 606, 609, 611 and 612 all use essentially the same procedure for sampling, sample extraction, and concentration. Thus a single sample may be used to measure the parameters within the scope of these methods.

Sample container materials, preservation techniques, and holding times are critical to the procedures and are specifically defined (Methods 601–613, 624, 625, 1624 and 1625). The design and operation of the purge-and-trap device in Methods 601–603, 624 and 1624, and the sample extraction procedures of Methods 604–613, 625 and 1625 are precisely defined as well.

In response to public comments, substantive revisions were made to allow more flexibility in the remaining parts of Methods 601–613, 624, 625, 1624 and 1625. In Methods 604–613, after the sample has been extracted, the analysts are now free to choose a technique to concentrate the extract. The same flexibility is provided for selecting the GC or HPLC configurations (column packings, operating conditions, and detectors). When analysts use concentration techniques or chromatographic configurations other than those described in the test procedures, their approaches must meet

they were tested, and sample matrices which show labeled compound recoveries significantly different from recoveries of these compounds from reagent water are diluted with reagent water to bring these recoveries into the expected range.

It is also important to note that the studies provide a strong basis for setting control limits which represent a range of acceptability. The studies show that most laboratories will do far better, especially on a single-operator, single-laboratory basis. Other performance studies, completed since the inter-laboratory analyses, incorporate too much flexibility to be directly analogous to EPA's collaborative test of the methods. However, they appear to confirm the assumption that most laboratories will exceed the minimum standards and indicate that method variability will be well within the range of the control limits.

The final specifications derived for all of the organics methods (except 603) were the result of a statistical analysis of the data from the multi-laboratory studies. These specifications adopt initial precision and accuracy for all methods. For start-up calibration verification, they specify control limits for Methods 601, 602, 624, 1624, 625 and 1625. For on-going accuracy, they specify control limits for recovery of pollutant spikes for Methods 601–613, 624, and 625, and for recovery of labeled compound spikes for Methods 1624 and 1625. The methods allow for simultaneous testing of all the parameters listed in each method.

In theory, a problem could arise from simultaneous tests for numerous compounds. The control limits have been calculated to allow only a 5% likelihood that a result that exceeds the limits for each compound is merely a statistical fluctuation (rather than actual error). However, the chance of "statistical error" rises with the number of compounds being tested.

EPA has corrected for this possibility in several ways. First, most users will not apply each analysis to all parameters simultaneously; thus they will have a greater chance of passing all test criteria. Second, in order to allow for simultaneous testing of all parameters in a given method, the specifications for accuracy and precision have either been broadened, or a re-test has been allowed, or both. The technique of using a re-test was chosen because a one-test-only specification which allowed for simultaneous testing of a large number of parameters would be so broad as to have little meaning. The provision for a re-test preserved a meaningful specification while allowing for simultaneous testing of all parameters. If a laboratory fails the re-test as well as the initial test, the likelihood of "statistical error" is extremely low (5% times 5%, i.e., .0025 for a given compound). Third, when a re-test is required, it need only be performed on the particular compounds which failed the initial test. Finally, the control criteria for Methods 1624 and 1625—those most likely to be simultaneously used on many compounds—were determined based on the 99% confidence level.

As a voluntary guide to laboratories practicing a given method, the following Exhibit 1 gives suggested numbers of first pass test criteria failures which are unlikely if the laboratory is satisfying the probability based quality control specifications. It assumes all parameters in a given method are tested simultaneously. The Exhibit indicates the maximum number of parameters for which each method can be used simultaneously. The two right-hand columns indicate a certain number of unacceptable results. If the analyst finds that number, or a greater number, of unacceptable results, he may conclude that the entire analysis is flawed. If so, it may be more efficient to repeat the entire analysis than to re-examine only the compounds which exceed the control limits.

EXHIBIT 1.—SUGGESTED MAXIMUM NUMBER OF TEST CRITERIA FAILURES WHICH JUSTIFY REPEATING ENTIRE ANALYSIS

Method	Number of simultaneous parameters	Number of test criteria failures	
		Start-up [1]	On-going [2]
601	29	7	4
602	7	3	2
603/605	2	2	2
604	11	4	3
606	6	3	2
607	3	2	2
608	25	6	4
609	4	3	2
610	16	5	3
611	5	3	2
612	9	4	3
613	1	2	1
624	31	7	5
625	61	11	7
1624	66	12	7
1625	151	7	5

[1] Based on twice the number of parameters being tested since both accuracy and precision are being evaluated.
[2] Based on the number of parameters being tested.

Section 8 of each method defines acceptable analytical performance limits for the GC, HPLC, and GC/MS test procedures (Methods 601–613, 624, 625, 1624, and 1625). These acceptable performance limits are also specified in Footnote 7 to Table IC, "List of Approved Test Procedures for Non-Pesticide Organic Compounds," and Footnote 7 to Table ID, "List of Approved Test Procedures for Pesticides." System performance is acceptable only when the average recoveries and standard deviations of spikes of the pollutants of interest into reagent water meet these performance standards. Where large numbers of parameters are being analyzed (see Exhibit 1 above), there is an increased chance that at least one parameter will fail for either average recovery or standard deviation limits based purely on chance. Where such failure occurs, the spiking and recoveries must be repeated, but only for the failed parameters. Repeated failure confirms a general problem with the analytical measurement system. When such failed recoveries are experienced the system is judged to be out-of-control for the failed parameter. Thus, the results for the failed parameters in unspiked samples are suspect and cannot be reported to show regulatory compliance.

The acceptance criteria for spikes into samples for each parameter were calculated to include both an allowance for error in prior measurement of the background and another allowance for error in prior measurement of spike concentrations. The calculation assumed a spike-to-background ratio of 5 to 1. Thus such error will be accounted for to the extent the analysts' spike-to-background ratio approaches 5 to 1. In many cases this allows analysts a greater margin of error than should actually be expected. This is because the calculation assumes that two prior errors are cumulative, ignoring the degree to which they actually cancel each other out.

Today's final test procedures represent an effort to provide the maximum uniformity that is practical for a wide cross-section of classes of chemical compounds. They will be continually reevaluated for their general applicability to complex wastewater matrices.

The substantive revisions made in the GC, HPLC, and GC/MS methods in response to comments are discussed in the public participation section of this preamble. Three of the most significant changes include: (1) Addition of a confirmatory column to Method 602; (2) deletion (from 613) of the gas chromatographic/electron capture (GC/EC) test procedure for screening for 2,3,7,8-TCDD, and (3) revision of Methods 613 and 625 to show that Method 625 may be used whenever screening for 2,3,7,8-TCDD is required. The full text of the approved GC, HPLC, and GC/MS test procedures are being printed in Appendix A of this regulation.

The GC, HPLC, and GC/MS test procedures are now cited in the regulations in the new Table IC, "List of Approved Test Procedures for Non-Pesticide Organic Compounds," and Table ID, "List of Approved Test Procedures for Pesticides."

C. ICP Test Procedure

The ICP test procedure is cited in the regulation as an additional analytical option for trace metal analysis in the new Table IB, "List of Approved Inorganic Test Procedures."

The ICP test procedure, Method 200.7, has been changed only slightly from the version proposed on December 3, 1979. EPA proposed that lithium and strontium be analyzed using the ICP test procedure, since these parameters could be analyzed using this method. Because EPA did not propose or develop accuracy or precision criteria for these parameters, EPA is unable to approve the ICP test procedure for them. EPA is considering the ICP and other alternative test procedures in a separate rulemaking. In light of additional information received in the public comments showing good recoveries for antimony and adequate recoveries for thallium by the proposed test procedure, both of these metals have been added to the scope of the ICP test procedure. Also in response to public comments the detection limit for silica has been doubled and the wavelengths of the metal are now given to the third decimal. In section 3 of the ICP test procedure a new definition for "Quality Control Sample" has been provided for clarification, and a new section on safety has been added to alert the analyst to the hazards of the toxic reagents and pollutants involved. Other revisions made in response to comments are discussed in the public participation section of this preamble. The full text of the ICP procedure is printed as Appendix C to this regulation.

D. $CBOD_5$ Test Procedure

The final test procedure for $CBOD_5$ is essentially the same as that proposed. See Section III–D, above. EPA's proposed test procedure was taken from a draft *Standard Methods* test procedure for $CBOD_5$.

The final method language is the same as the language now included in the 15th edition of *Standard Methods*. This has required minor changes from the wording of the proposal, but no substantive changes were required.

E. Table II: Required Containers, Preservation Techniques, and Holding Times

Table II in Section 136.3(e) now restricts the materials of which sample containers can be made, and specifies the procedures by which samples are to be preserved. Table II also limits the maximum time for which samples may be held from the the time of sampling until they are analyzed. Table II has been restructured in this final regulation to correlate with the parameters in the new Tables IA, IB, IC, ID, and IE in Section 136.3(a). Table II allows cross-reference between the container, preservative, and holding times and the individual parameters in Tables IA, to IE.

In response to comments, several changes were made in Table II of the final regulations for prescribed container materials, preservation requirements, and holding times of wastewater samples. Where supported by comments, changes were made primarily in holding times. In response to comments, EPA has adopted the requirement that some samples be analyzed immediately, to avoid sample degradation. This would be as soon as the sample is collected and labelled, generally within 15 minutes. Longer holding times are generally not appropriate where the sample may quickly degrade. However, a longer time period may be justified under the variance procedure. Exhibits 3 and 4, below, show that for organic compounds and pesticides, the holding times were generally extended from 30 days after extraction to 40 days after extraction. Changes were also made to enable a single sample to be used for analyses of extractable organics and of pesticides. This was a step towards the goal of uniformity, sought by EPA and by the commenters.

Table II as promulgated also allows a variance to holding times under § 136.3(e). Analysts may exceed the holding times if they have data on file to show that the specific types of samples are stable for a longer time and if they receive a variance from the Regional Administrator.

No changes were made for container materials, preservation requirements, or holding times in final Table II from the proposed requirements for the biological parameters listed in Table IA, or the radiological parameters listed in Table IE. Changes which were made in Table II for inorganic parameters listed in Table IB, organic parameters listed in Table IC, and pesticide parameters listed in Table ID are summarized in the following Exhibits 2, 3, and 4, of this preamble. Proposed and final container materials, preservation requirements, and holding times in Exhibits in 2, 3, and 4 are given only for the affected pollutant parameters in Tables IB, IC and ID of the regulation.

EXHIBIT 2.—CHANGES MADE IN TABLE II FOR TABLE IB PARAMETERS

Parameter	Requirement	Change	
		From (proposed)	To (final)
Chlorine residual	Holding time	2 hours	Analyze immediately.
Cyanide	Preservative	$Na_2S_2O_3$	Ascorbic acid.
pH	Holding time	2 hours	Analyze immediately.
Chromium VI	Holding time	48 hours	24 hours.
Mercury	Preservative	0.05% $K_2Cr_2O_7$	Delete.
Organic carbon	Preservative	None	Add: HCl or H_2SO_4 to pH<2.
Dissolved oxygen probe	Holding time	1 hour	Analyze immediately.
Winkler	Preservative		Add: Store in dark.
Phenols	Container	P and G	G only.
Residue, total	Holding time	14 days	7 days.
Residue, filterable	Holding time	14 days	7 days.
Residue, settleable	Holding time	7 days	48 hours.
Sulfide	Preservative		Add: NaOH to pH>9.
	Holding time	28 days	7 days.
Sulfite	Holding time	48 hours	Analyze immediately.
	Preservative	Cool to 4 °C	None required.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MEMORANDUM

OFFICE OF WATER

SUBJECT: **Recommended Approved Modifications to EPA Method 625**

FROM: Richard Reding, Chief
Engineering & Analytical Support Branch, EAD, OST

TO: Quality Assurance Managers
ATP Coordinators
NPDES Coordinators

DATE: November 1, 2006

The 304(h) methods branch recommends allowing several modifications to EPA Method 625 for environmental permitting and compliance monitoring under the EPA's Clean Water Act (CWA) programs. This memorandum does not address laboratory certification requirements that states have mandated.

The text in "Protocol for EPA Approval of Alternate Test Procedures for Organic and Inorganic Analytes in Wastewater and Drinking Water" Section 1.3.2 allows flexibility in the modification of "front end techniques" of the test method provided all criteria in this section and **all QC in the method are** met and documented. This protocol can be downloaded at http://www.epa.gov/waterscience/methods.

Recommendations on Method Modifications to EPA Method 625 when Capillary Columns are used:

1. **Combining sample extracts before analysis**

If the analytes can be reliably identified and quantified in the combined extracts, the extracts may be combined. If, however, the identification and quantitation of any analyte is adversely affected by another analyte, a surrogate, or an interferant, the extracts must be analyzed separately. If there is ambiguity, the extracts must be analyzed separately.

2. **Reverse order of pH extraction**

The pH extraction sequence may be reversed to better separate acid and neutral components. Neutral components may be extracted with either acid or base components.

Previously, neither of these modifications has been used with Method 625 primarily because of limitations of the resolving power of the packed columns used. In 1985, EPA Region 3 Central Regional Lab requested a modification to method 625 as an alternate test procedure (ATP). Although the approval was for limit use by EPA's Region 3, Central Regional Laboratory only, this modification has come to be used throughout the laboratory community (see attached memo).

Why allow these modifications? Following the base-neutral than acid extraction sequence of method 625 in some cases demonstrated the decomposition of some analytes under basic conditions. Organochlorine pesticides may dechlorinate; phthalate esters may exchange; phenols may react to form tannates. These reactions increase with increasing pH. Reversing the extraction pH sequence may better separate acid and neutral waste components.

Other Recommended Modifications to Method 625

A smaller sample volume may be used to minimize matrix interferences provided matrix interferences are demonstrated and documented.

Alternate surrogate and internal standard concentrations other than those specified in the method are acceptable provided that method performance is not degraded;

An alternate calibration curve and a calibration check other than those specified in the method;

A different solvent for the calibration standards to match the solvent of the final extract.

Other Method Flexibility News

We are revising the "Guidance on Evaluation, Resolution, and Documentation of Analytical Problems Associated with Compliance Monitoring" often referred to as the "Pumpkin Book". Many of the recommendations in the revised "Pumpkin Book" cover ways to mitigate matrix effects.

More explicit flexibility to make changes in approved methods without prior EPA approval is now described at 40 CFR Part 136.6. Such changes are only allowed if the modified method produces equivalent performance for the analyte(s) of interest, and the equivalent performance is documented. It is essential to consult the full text at 40 CFR 136.6 before undertaking method modifications.

Please feel free to forward this information. If you have any questions regarding this memorandum, please contact Lemuel Walker of EASB/EAD/OST by email at walker.lemuel@epa.gov.

cc Lemuel Walker
ATP Coordinator